Report for 2005TX197B: Spatial Patterns in Wetland Nutrient Biogeochemistry: Implications for Ecosystem Functions

Publications

- Articles in Refereed Scientific Journals:
    - Scott JT, Doyle RD, Back JA, and Dworkin SI (In-review) Elemental, isotopic, and enzymatic evidence for seasonally evolving phosphorus limitation in a nitrogen-fixing wetland periphyton community. Biogeochemistry.
    - Scott JT, McCarthy MS, Gardner WS, and Doyle RD (In-review) Alteration of inorganic N:P supply by ecosystem retetion and transformation of nitrogen in a constructed marsh. Biogeochemistry.
- Conference Proceedings:
    - Scott JT and Doyle RD. N2 fixation and the natural abundance of N isotopes in a wetland periphyton community. Society of Wetland Scientists South Central Chapter Meeting, October 2005, San Marcos, Texas.
    - Scott JT and Doyle RD. N2 fixation and the natural abundance of N isotopes in a wetland periphyton community. Society of Wetland Scientists International Cogress, July 2006, Cairns, Queensland, Australia.

Report Follows

Spatial Patterns in Wetland Nutrient Biogeochemistry: Implications for Ecosystem Functions

Thad Scott

Abstract

The role of nitrogen (N_2) -fixation in balancing N supply to a wetland periphyton mat was assessed by comparing measurements of primary production with elemental, isotopic, and enzymatic correlates. Measurements of primary production, N_2 fixation (acetylene reduction (AR)), phosphatase activity, C:N:P content of periphyton, and N isotopic composition of periphyton were made along a nutrient availability gradient in a freshwater marsh during May through September 2004. Periphyton primary production was correlated with inorganic N availability in the spring, but became more closely correlated (inversely) with phosphatase acitivity during the remainder of the summer. Nitrogen fixation and phosphatase activity in periphyton were negatively correlated with inorganic N and P availability, respectively. Elemental imbalance appeared to shift from N imbalance (C:N > 15 by mass) in spring to P imbalance in the summer (C:P ≥ 150 by mass). The percent contribution of N_2 fixation to total N uptake, determined from AR measurements and estimates of gross N assimilation, was highest in the spring (0 – 50 % in May 2004) and decreased throughout the summer (0 – 25% in July 2004 and 0 – 10 % in September 2004). However, N isotopic data suggested the sustained importance of an atmospheric N_2 source throughout the summer. This discrepancy is likely explained by the seasonal accumulation and retention of sequestered N supplies. When periphyton primary production was normalized to periphyton N content, production rates for the entire growing season were strongly inversely correlated with periphyton phosphatase activity ($r^2 = 0.78$). Results of the study suggest that N_2 fixation may adequately supplement community N supplies in wetland periphyton mats over short-time scales (weeks), thereby causing limitation by other elemental resources such as P.

Introduction

Primary production in freshwater aquatic ecosystems will proceed until limited by resource availability. In general, photoautotrophs become limited by either the availability of light energy or elemental resources (*e.g.* nitrogen (N) or phosphorus (P)). When elemental resources are sufficient, photoautotrophs will grow until their own biomass decreases the amount of available light energy or until nutrients have been exhausted. Schindler (1977) suggested that N resources of lakes could only be exhausted temporarily and therefore P would limit primary production in these systems over extended time scales. This hypothesis was based on the ability of planktonic heterocystous cyanobacteria to fix atmospheric N (N_2) at a rate sufficient to supplement the N supply to an ecosystem. Cyanobacterial N_2 fixation rates, and conditions controlling the establishment of cyanobacteria and initiation of N_2 fixation, have since been widely described for a variety of aquatic habitats (see reviews by Howarth et al. 1988a and 1988b) with the greatest attention given to planktonic systems (Vitousek 2002). Although Schindler's hypothesis (1977) did not hold true for estuarine and marine systems (due to trace element limitation of the nitrogenase enzyme; see Howarth et al. 1988b for details), "evolving" P-limitation in phytoplankton has often since been assumed for freshwater systems. However, the rate at which

N_2 fixation can supplement ecosystem N supply has not been rigorously tested, even for planktonic systems.

Howarth et al. (1999) derived a mechanistically-based simulation model to test the interacting effects of molybdenum-limitation and zooplankton grazing on cyanobacterial exclusion from estuarine phytoplankton. Although the model could accurately predict the timing and magnitude of N_2 fixing cyanobacterial blooms in lakes, the model did not include a mass balance term that would suppress N_2 fixation in response to N accumulation. In that and subsequent papers, the authors suggested the need to refine the model to include the inherent feedback of N accumulation in the N_2-fixing community (Howarth 1999, Vitousek et al. 2002). Although many studies have identified dissolved inorganic N (DIN) concentrations at which cyanobacterial N_2 fixation diminishes (see Horne et al. 1979, Doyle and Fisher 1994, and Scott et al. 2005), few studies, if any, have characterized how feedback conditions such as N accumulation might suppress N_2 fixation or even stimulate P-limitation of primary production.

In wetland and shallow lake environments (lentic ecosystems), periphyton communities can dominate microbial primary production. In particular, floating periphyton mats or "metaphyton" (Stevenson 1996), which form by fragmentation of epipelic, epilithic, or epiphytic communities, can be highly productive and radically alter ecosystem nutrient cycling (Wetzel 1996). These communities often efficiently retain and recycle sequestered nutrient stocks. However, nutrient-limitation of periphyton primary production has been widely demonstrated in enrichment experiments utilizing both whole-system (McDougal et al. 1997, Havens et al. 1999, McCormick et al. 2001) and diffusion substrate approaches (Fairchild et al. 1985, Scott et al. 2005). Many of these studies demonstrated periods of N and/or P-limitation, and/or periods of N+P co-limitation. Co-limitation describes a scenario whereby only combined enrichments of N and P (and possibly other micronutrients as well) resulted in growth stimulation. When N or both N and P are in low supply and cyanobacteria are present, N_2 fixation should commence when the energetic costs of N-limitation (*i.e.* unrealized primary production) exceed the energetic cost of N_2 fixation. When N stocks have been replenished, N_2 fixation will cease as its energetic cost begins to exceed the potential production achievable solely with DIN (Tyrell 1999).

To our knowledge, no studies have assessed the impact of N accumulation derived from N_2 fixation in lentic periphyton communities. In fact, few studies on periphyton nutrient limitation have included N_2 fixation measurements at all. Scott et al. (2005) found that periphyton were increasingly N-limited and exhibited increasing N_2 fixation potential along the longitudinal axis of a created wetland. However, that study did not attempt to quantify N accumulation within the periphyton community. In a Florida Everglades periphyton community, Inglett et al. (2004) found that N accumulated with increasing N_2 fixation throughout the summers of 1998 and 1999 and that periphyton N content was always greatest in September when N_2 fixation rates began to decline. Although these results suggest that N_2 fixation may have been sufficient to balance periphyton N content within one summer season, this study did not assess the limitations to periphyton primary production over any time scale.

Interestingly, N_2-fixing periphyton communities are conspicuous in tropical and sub-tropical marshes, such as the Florida Everglades, which are generally considered strongly P-limited. For instance, Rejmánková et al. (2000) found that P enrichment increased periphyton primary

production but that N enrichment did not change periphyton primary production in three marshes of northern Belize. In two of the three marshes where P enrichment increased primary production, a simultaneous increase in N_2 fixation rates and decrease alkaline phosphatase activity (APA) were observed. This suggests that P availability may have limited primary production but that P enrichment may have resulted in an N deficiency. Additionally, periphyton N content was generally higher in the third marsh where N_2 fixation and APA did not change when enriched with P, than it was in the two marshes where N_2 fixation and APA responded to P enrichment. This further suggests that N rich periphyton did not waste energy on N_2 fixation to bring N supply into balance, which is in general agreement with trends observed in phytoplankton (Tyrell 1999). Considered collectively, the results of Rejmánková et al. (2000) suggest that cyanobacterial N_2 fixation could have been sufficient to maintain N balance during the 10 day experimental period but the process was only initiated under relatively low periphyton N content.

If we are to understand the role of nutrient limitations to primary production in any ecosystem, it is imperative that we have a complete understanding of the role of biological N_2 fixation in ecosystem production (Vitousek 2002). Aquatic ecosystems, and periphytic communities in particular, provide a unique opportunity to study the role of N_2 fixation in alleviating N-limitations to community production. In this study, we attempted to quantify the seasonal role of periphytic N_2 fixation in balancing community N stocks and inducing P-limitation to primary production. Specifically, the objectives of the study were to: 1) quantify periphyton primary production along a nutrient availability gradient, 2) quantify rates of biological N_2 fixation relative to periphyton primary production and nitrogen content, 3) quantify periphyton phosphatase activity relative to primary production, and 4) describe both short-term (instantaneous) and long-term (seasonal) limitations to periphyton primary production.

Materials and methods

Site Description

The study was conducted at the Lake Waco Wetland (LWW) complex, near Waco, Texas, USA (Figure 1). The complex is an 80-ha off-channel constructed marsh that receives water pumped from the North Bosque River. Water meanders through five wetland cells before flowing back into the North Bosque River. The average depth of the wetland is approximately 0.5 meters and the hydraulic residence time ranges between 10 to 30 days. During the course of

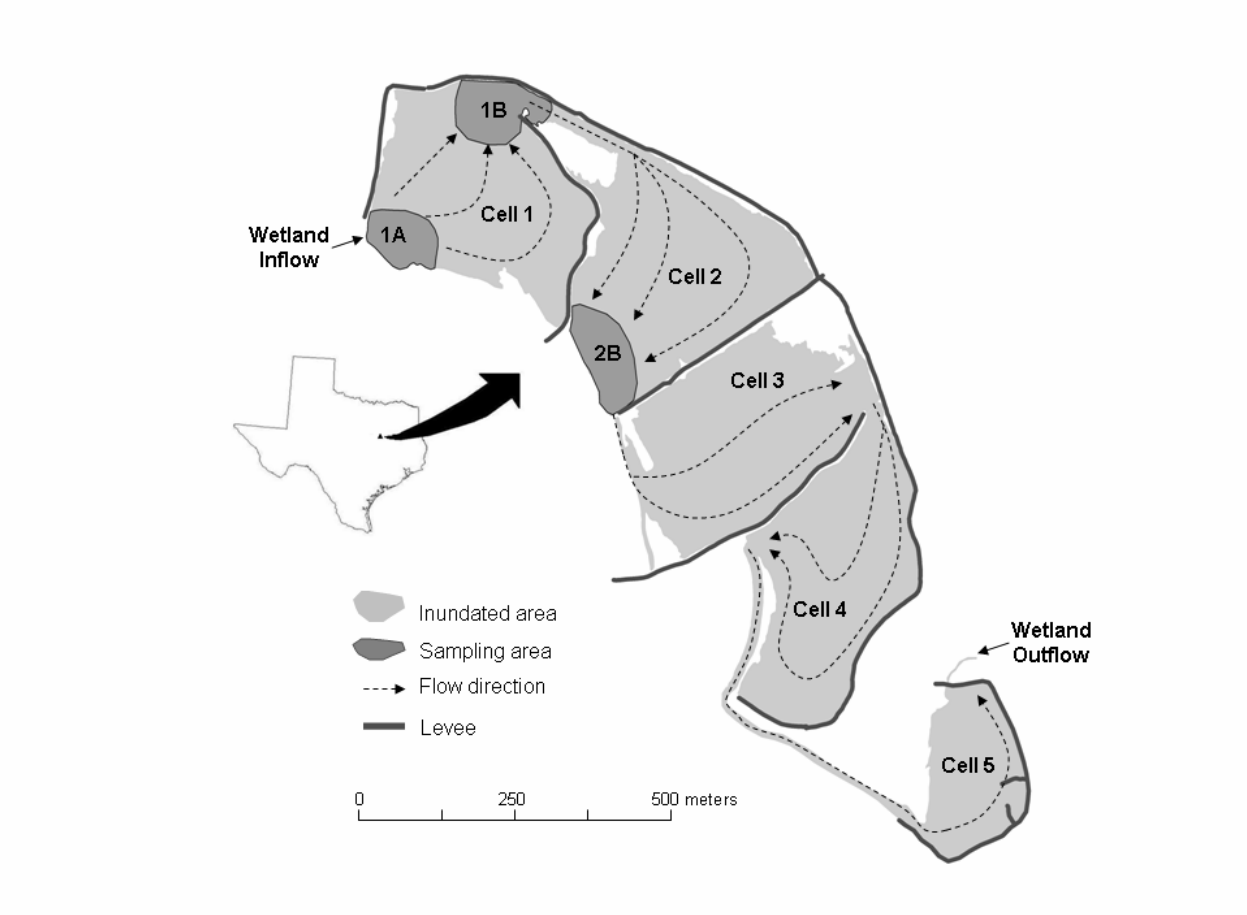

Figure 1. Lake Waco wetland, near Waco, Texas, USA. Periphyton samples were collected in May, July, and September 2004 from random locations with areas 1A, 1B, and 2B. Water chemistry was measured biweekly from May – September 2004 in each sampling area.

this study, the mean pumped inflow rate was 6.0 million gallons per day (range = 0 to 11 MGD) and the average hydraulic residence was 17.5 days.

A floating periphyton community generally occurs in the relatively deep (≥ 1m), open-water areas of this wetland. This community is comprised of green algae (*Hydrodiction* sp., or *Cladophora* sp.) and a consortium of attached microbiota including diatoms, cyanobacteria, and heterotrophic bacteria (Scott, unpubl.). Scott et al. (2005) demonstrated that the periphyton community in the downstream areas of the LWW was strongly N-limited and exhibited high N_2 fixation potential. Furthermore, periphytic N_2 fixation was light-dependent and generally carried out by heterocystous cyanobacteria or diatoms with cyanobacterial endosymbionts such as *Epithemia adnata* and *Rhopalodia gibba*. Near the inflow however, periphyton were either co-limited by N and P or not limited by nutrients at all. N_2 fixation potential in the periphyton community near the inflow was always zero. Given these trends, the LWW provides a well documented gradient where available inorganic nutrients decrease in the downstream direction. Therefore, the LWW exhibits ideal conditions to test hypotheses regarding evolving P-limitation and the role of N_2 fixation in balancing periphyton N content.

Periphyton mat samples were randomly collected from areas 1A, 1B, and 2B of the LWW (Figure 1) in May, July, and September 2004. These sampling areas were positioned along the gradient of nutrient availability which corresponds to the flow path of water (Figure 1; see Scott et al. 2005). Approximately 400 cm^2 of periphyton mat were harvested, placed into a plastic container with site water, and transported to the laboratory. Eight replicate samples were collected from each area on all dates. In the laboratory, periphyton mat samples were subsampled for measurements of primary production, N_2 fixation via acetylene reduction, phosphatase activity, C, N, and P content, and $\delta^{15}N$ composition. In addition to periphyton samples, four liters of water were collected on each sampling date to use as incubation water in laboratory bioassays for primary production and N_2 fixation. Water samples were also collected in each sampling area on a biweekly basis during the course of the study for water chemistry analysis.

Primary Production

Periphyton primary production was determined by measuring the rate of O_2 production in high-light, low-light, and dark incubations (Wetzel and Likens 2000). Three small subsamples (≤ 1 cm^2) were cut away from each periphyton sample. Two portions were transferred into transparent BOD bottles with 300 ml site water and the third portion was transferred into an opaque BOD bottle with 300 ml site water. The concentration of dissolved O_2 was determined to a precision of 0.1 ppm before incubation with a YSI 5000 dissolved O_2 meter. Prior to addition to BOD bottles, dissolved O_2 concentration of incubation water was reduced to ~ 3.0 ppm by bubbling with N_2 gas amended with 350 ppm CO_2. All BOD bottles were placed in a water bath incubation set to *in-situ* temperature conditions. One transparent bottle was incubated under high-light intensity (~ 390 - 460 μmol m^{-2} s^{-1}) and the other under low light intensity (~ 45 - 55 μmol m^{-2} s^{-1}). Samples were incubated until dissolved O_2 concentrations increased by ~ 1 to 1.5 ppm in low light incubations (~ 1-4 hours). Final dissolved O_2 concentration, incubation time, and photon flux density were recorded at the end of incubation. The photon flux density encountered by each incubation bottle was determined by measuring irradiance at the location of each bottle in the water bath with a LI-COR LI-250 light meter equipped with a spherical sensor. In addition to periphyton samples, samples containing only site water from each sampling location were incubated in duplicate at each light level to account to photosynthesis and respiration by plankton.

Following incubation, the samples were filtered onto a pre-washed, -dried, and –weighed glass fiber filter then oven-dried at 60 °C overnight. The dry weight of sample was calculated as the final weight of the sample and filter minus the original filter weight. Gross photosynthesis for samples at each light level was calculated as:

$$GP = \frac{\left[\left(O_{2e} - O_{2r}\right) \times 0.375 \times 0.3\right]}{\left(PQ\right) \times \left(t\right) \times \left(DW\right)}$$

where GP is the rate of gross photosynthesis (mg C g DW^{-1} h^{-1}), O_{2e} is the change in dissolved O_2 concentration (mg O_2 L^{-1}) in the transparent bottle over time t, O_{2r} is the change in dissolved O_2 concentration (mg O_2 L^{-1}) in the opaque bottle over time t, PQ is the photosynthetic quotient (dimensionless constant = 1.2; see Wetzel and Likens 2000), and DW is the dry weight of the sample in grams. The constant 0.375 represents the ratio of carbon fixed to oxygen generated

during photosynthesis, and the constant 0.3 was the incubation volume in liters. Low-light photosynthetic rates were standardized to the average low-light condition by dividing the photosynthetic rate by the measured incubation light intensity and multiplying by the average low-light incubation condition (40 μmol m^{-2} s^{-1}). Standardized low-light gross photosynthesis was multiplied by 8 hours, light-saturated (determined from high-light incubations) gross photosynthesis was multiplied by 4 hours, and these rates were summed to derive an estimate of daily periphyton primary production expressed as mg C g DW^{-1} day^{-1}.

N_2 fixation

Acetylene reduction was used to estimate the rate of N_2 fixation in periphyton samples. In this assay, acetylene is reduced to ethylene by the nitrogenase enzyme at a rate proportional to the reduction of N_2 to NH_4^+ (Flett et al. 1976). The measurement is indicative of N_2 fixation in a sample at a given point in time (*i.e.* instantaneous N_2 fixation). In the laboratory, three small subsamples ($\leq$ 1 cm^2) were cut away from each periphyton sample and placed into Popper Micromate syringes with 30 ml site water. One syringe was wrapped in foil for dark incubation while the other two were used for high-light and low-light incubations. Five milliliters of acetylene gas were injected into each syringe which was then gently mixed to allow rapid dissolution of acetylene. Syringes were incubated as described above for primary production. At the end of incubation, 15 ml of air were drawn into each syringe which was then vigorously agitated to establish equilibrium conditions of gases between aqueous and vapor phases. Water and vapor volumes were recorded to account for partitioning between phases and ethylene concentration of the vapor was determined using a Carle AGC Series gas chromatograph (GC). The GC was equipped with a flame-ionization detector and a 1.8 m column packed with 80% Porapack N and 20% Porapack Q (80/100 mesh). The column temperature was 70 °C, helium was used as the carrier gas, and 10 ppm ethylene standards were used to calibrate the instrument daily. For each light level (dark, low-light, high-light), the hourly ethylene production rate was converted to an hourly N_2 fixation rate assuming that the production of 3 μmol ethylene was equivalent to the fixation of 1 μmol N_2 (Flett et al. 1976). Low-light N_2 fixation rates were standardized to average low-light conditions and used with light-saturated N_2 fixation rates to derive estimates of daily periphyton N_2 fixation (μg N g DW^{-1} day^{-1}) following the same method described above for primary production.

Phosphatase Activity

Phosphatase activity may be used as an indicator of P-limitation in periphyton. Organisms will increase phosphatase production in an attempt to increase P availability. In this study, phosphatase activity in periphyton was measured fluorometrically using methylumbelliferone phosphate (MUP) as a substrate. In the presence of phosphatase enzymes the phosphate group on MUP is hydrolyzed yielding methylumbelliferone (MU). MU fluoresces when irradiated at 365 nm wavelength. In samples saturated with MUP, the rate of increasing fluorescence is proportional to the rate of MU production, and subsequently, phosphatase activity (Petterson 1980). Periphyton subsamples were transferred into 15 ml culture tubes with 9 ml 1.2 % TRIS buffer (pH 8.3). One milliliter of 10^{-4} mol L^{-1} MUP was added to each tube and samples were mixed gently. Samples were incubated at room temperature under ambient indoor lighting. Fluorescence was measured after 5, 15, and 45 minutes on a Turner 10 AU fluorometer calibrated with 50, 100, 250, 500, and 1000 ppb MU standards. Dry weight of all samples was

determined as previously described and phosphatase activity expressed as nmol P_{ase} g DW^{-1} min^{-1}.

Periphyton Elemental and Isotopic Composition

A subsample of each periphyton sample was oven-dried overnight at 60 °C and ground to a fine powder for determination of C, N, and P content and N isotopic composition. C and N content were determined simultaneously using a Thermo Finnigan FlashEA 1112 elemental analyzer. Phosphorus content was determined colorimetrically on a Lachat Quickchem 8500 following a 3 hour digestion in concentrated H_2SO_4 at 370 °C (Clesceri et al. 1998). Nitrogen isotopic composition was measured using a continuous flow isotope ratio mass spectrometer connected to a Carlo Erba NA1500 elemental analyzer. Measured $^{15}N/^{14}N$ ratios are expressed in delta notation (δ):

$$\delta^{15}N_{sample} = \left[\left(\frac{R_{sample}}{R_{air}}\right) - 1\right] \times 1000$$

where $\delta^{15}N_{sample}$ is the isotopic composition of the sample expressed in units of per mil (‰), R_{sample} is $^{15}N/^{14}N$ ratio measured in the sample, and R_{air} is the $^{15}N/^{14}N$ ratio of air.

Contribution of N_2 fixation to total N uptake

We estimated the contribution of N_2 fixation to total N uptake by periphyton using two separate methods: 1) acetylene reduction measurements with gross N assimilation estimates, and 2) $\delta^{15}N$ composition of periphyton. Estimates from acetylene reduction assays were derived by dividing the rate of N_2 fixation measured by acetylene reduction by the rate of gross N assimilation. Gross N assimilation was estimated by multiplying the rate of primary production of a sample by its measured ratio of N:C. The quotient of N_2 fixation, expressed as mg N g DW^{-1} h^{-1}, and gross N assimilation, also expressed as mg N g DW^{-1} h^{-1}, is the percent contribution of N_2 fixation to total N uptake.

The use of $\delta^{15}N$ as an indicator of the contribution of N_2 fixation to total N uptake is based on the general isotopic difference between atmospheric N_2 and DIN. Atmospheric N_2 has a constant N isotopic composition. When air is used as the reference standard in mass spectrometry (as is usually done for N), $\delta^{15}N$ of atmospheric N_2 is 0 ‰. The isotopic composition of DIN in freshwaters however, is usually relatively heavy ($\delta^{15}N \approx 5 - 15$ ‰; Heaton 1986). Because N isotopic composition of autotrophs will usually reflect the isotopic composition of their inorganic N source (Lajtha and Marshall 1994), it is often possible to estimate the relative contribution of DIN and atmospheric N_2 to autotrophic communities that can utilize either N source by measuring the N isotopic composition of the community itself (Gu and Alexander 1993; France et al. 1998).

For our purposes, a two-end member mixing model was developed to predict the relative contribution of N_2 fixation to total N uptake by periphyton. The model was constructed as follows:

$$\delta^{15}N_{periphyton} = \left[\left(C_{N_2}\right)\times\left(\delta^{15}N_{N_2} + f_{N_2}\right)\right] + \left[\left(C_{DIN}\right)\times\left(\delta^{15}N_{DIN} + f_{DIN}\right)\right]$$

where $\delta^{15}N_{periphyton}$ is the N isotopic composition of periphyton, C_{N_2} is the percent contribution of atmospheric N_2 to total nitrogen uptake by periphyton, $\delta^{15}N_{N_2}$ is the isotopic composition of N_2 (0 ‰), and f_{N_2} is the fractionation value associated with N_2 fixation (assumed to be -2.0 ‰ based on the value of fractionation reported for cyanobacterial cultures using N_2 as their sole source of N (Gu and Alexander 1993)), respectively. C_{DIN} is the percent contribution of DIN to total N uptake which was set equal to $1 - C_{N_2}$ in this mixing model. $\delta^{15}N_{DIN}$ is the isotopic composition of DIN in waters flowing into the LWW (assumed to be 9.7 ‰; from Dworkin 2003), and f_{DIN} is the fractionation value associated with DIN uptake. In general, fractionation with DIN uptake will occur only when DIN is in relatively abundant supply and should approach zero when DIN availability is at limiting or near-limiting levels (see Fogel and Cifuentes 1993). Because DIN has been shown to limit periphyton production in the LWW (Scott et al. 2005), f_{DIN} was assumed to be 0 ‰ in this study.

Periphyton $\delta^{15}N$, along with the previously stated assumptions, were used to solve the mixing model for the percent contribution of N_2 fixation to total N uptake (C_{N_2}). An important assumption inherent within the model was that N was not efficiently retained and recycled within the periphyton community. Predicted values of C_{N_2} calculated by the model were compared to estimates of the percent contribution of N_2 fixation to total N uptake derived from acetylene reduction assays and N uptake estimates using linear regression analysis in Sigma Plot 9.0 (Sigma Plot 2005).

Water Chemistry

Biweekly water chemistry samples were collected in acid-washed 1 liter polyethylene bottles and returned to the laboratory for analysis of nitrite-nitrogen plus nitrate-nitrogen (NO_2-N+NO_3-N), ammonia-nitrogen (NH_3-N), and soluble reactive phosphorus (SRP). NO_2-N+NO_3-N was determined colorimetrically on a Beckman DU 650 spectrophotometer following cadmium reduction (Clesceri et al. 1998). NH_3-N and SRP were also determined colorimetrically using the phenate and molybdenum blue methods, respectively (Clesceri et al. 1998). Water temperature, specific conductance, and pH were measured during sample collection with a YSI 6600 multiparameter datasonde.

Results

Water Chemistry

Average water chemistry conditions observed during the study are provided in Table 1. Conductivity was generally greatest at site 1A (wetland inflow), diminished at site 1B, and lowest at 2B, except in September when this pattern was reversed. Both NO_2-N+NO_3-N and NH_3-N followed a similar pattern of decreasing concentration along the flow path of water. However, this pattern was not observed in SRP. In May and July, SRP concentrations were generally similar amongst all sites. In September, average SRP concentration was highest at site 1B, followed by sites 1A then 2B. However, all differences observed in SRP concentrations

between sites were minor when compared to differences observed in dissolved inorganic nitrogen (DIN = NO_2-N+NO_3-N + NH_3-N). The ratio of DIN:SRP consistently decreased along the flow path of water during all months.

Periphyton Primary Production and Enzyme Activity

Periphyton primary production did not follow a consistent pattern among sites and dates (Table 2). In May, production was highest at site 1A, lower at site 1B, and lowest at site 2B.

Table 1. Water chemistry values for sites on each sampling date (mean ± SD; n=2 for all events).

Site	Water Temp(° C)	Spec Cond (µS cm^{-1})	pH	NO_2-N + NO_3-N (ppb)	NH_4-N (ppb)	DIN (ppb)	SRP (ppb)	DIN:SRP
May								
1A	25 ± 2	736 ± 59	7.8 ± 0.0	194 ± 6	22 ± 1	217 ± 5	4 ± 3	84 ± 69
1B	25 ± 3	715 ± 51	7.9 ± 0.2	44 ± 29	19 ± 6	63 ± 23	6 ± 4	14 ± 12
2B	26 ± 3	642 ± 65	7.8 ± 0.2	6 ± 2	14 ± 11	20 ± 10	5 ± 4	5 ± 2
July								
1A	30 ± 1	708 ± 344	8.0 ± 0.3	227 ± 136	69 ± 4	296 ± 132	8 ± 4	38 ± 4
1B	27 ± 0	665 ± 344	8.0 ± 0.3	42 ± 8	44 ± 27	87 ± 19	11 ± 8	12 ± 10
2B	29 ± 0	592 ± 399	8.0 ± 0.7	5 ± 3	15 ± 3	21 ± 1	4 ± 3	7 ± 5
September								
1A	26 ± 0	490 ± 12	8.1 ± 0.3	210 ± 89	74 ± 20	284 ± 69	3 ± 0	30 ± 115
1B	23 ± 1	501 ± 83	7.8 ± 0.6	100 ± 112	14 ± 13	115 ± 125	8 ± 5	12 ± 10
2B	24 ± 1	507 ± 83	7.8 ± 1.1	6 ± 3	8 ± 7	15 ± 4	1 ± 0	11 ± 4

However, this trend disappeared in July when highest rates were observed at site 1B, followed by 1A then 2B. Site 1B remained the most productive site in September, but site 2B displayed higher primary production than site 1A during this month. N_2 fixation measured using acetylene reduction (AR) was not detected at site 1A on any sampling event, but was always measurable at sites 1B and 2B (Table 2). N_2 fixation measured by AR was always light-dependent (Figure 2). Furthermore, the N_2 fixation rate was negatively correlated with average DIN concentration in the water column (Figure 3A) which resulted in a consistent spatial pattern of decreasing DIN and increasing periphyton N_2 fixation along the flow path of water. Phosphatase activity was always greatest at site 2B followed by site 1A then site 1B, except in September when phosphatase activity at site 1A exceeded that observed at site 2B (Table 2). In general, phosphatase activity was negatively correlated with SRP concentration in the water column (Figure 3B).

Periphyton Elemental and Isotopic Composition

The elemental composition of periphyton mats exhibited both spatial and temporal heterogeneity (Table 2). Carbon content at all sites was relatively low in May but generally increased through July and September. The only exception was at site 1A where C content appeared to slightly decrease from July to September. Nitrogen content followed a temporal pattern almost identical to C. Nitrogen content increased throughout the summer at all sites except 1A where N content was greatest in July but slightly decreased by September. Insufficient sample was collected for the determination of P content in the May samples. Data from the remainder of the summer show that periphyton P content was greater in July than in September for all sample sites. Elemental composition of periphyton did not appear to follow a spatial pattern similar to the distribution of dissolved inorganic nutrients except in September when periphyton C content was negatively correlated with mean DIN and periphyton P content was positively correlated with mean SRP (Tables 1 and 2). The carbon to nitrogen ratio (C:N) of periphyton was consistently between 12 and 15 except at sites 1A and 1B in May where values ranged between 18 and 21 (Figure 4). The ratio of carbon to phosphorus (C:P) and nitrogen to phosphorus (N:P) tended to exhibit more heterogeneity. In July, C:P and N:P were similar at sites 1A and 1B but higher at site 2B (Figure 5). This difference was due to diminished

Table 2. Primary production, enzyme activities, elemental composition, and isotopic composition of periphyton community for sites on each sampling date. All values are mean ± SD. For all values, n=8 except where indicated parenthetically.

Month / Site	Primary Production (mg C g DW^{-1} day^{-1})	Acetylene Reduction[1] (μg N g DW^{-1} day^{-1})	Phosphatase Activity (nmol P_{ase} g DW^{-1} min^{-1})	Carbon (wt. %)	Nitrogen (wt. %)	Phospho (wt. %)
May						
1A	39.0 ± 12.0 (7)	BDL[2]	60.5 ± 16.6	20.9 ± 3.3	1.21 ± 0.31	IS[3]
1B	22.0 ± 11.7	42.6 ± 33.6	41.6 ± 21.2 (7)	22.0 ± 3.2	1.08 ± 0.20	IS[3]
2B	10.6 ± 3.2	221.7 ± 38.1	104.4 ± 24.3 (4)	21.0 ± 1.7	1.41 ± 0.12	IS[3]
July						
1A	65.0 ± 23.8	BDL[2]	23.9 ± 12.6 (7)	23.3 ± 3.9	1.86 ± 0.45	0.26 ± 0
1B	82.3 ± 36.2 (7)	67.8 ± 106.3 (7)	14.2 ± 5.1 (7)	26.4 ± 2.6 (7)	1.93 ± 0.51 (7)	0.27 ± 0 (7)
2B	41.4 ± 16.3	384.9 ± 284.8	76.4 ± 33.8	24.3 ± 1.3	1.80 ± 0.15	0.18 ± 0
September						
1A	27.1 ± 10.5 (7)	BDL[2]	95.7 ± 50.8	19.9 ± 2.1	1.49 ± 0.20	0.10 ± 0
1B	107.3 ± 46.5	6.1 ± 6.3	31.9 ± 15.1	27.6 ± 4.1	2.28 ± 0.53	0.21 ± 0
2B	42.2 ± 6.7	60.7 ± 32.6	77.2 ± 25.0	32.1 ± 1.8	2.02 ± 0.27	0.16 ± 0

[1]N_2 fixation determined from acetylene reduction assays with intact periphyton.
[2]BDL – below detection level (1 ppm ethylene).
[3]IS – Insufficient sample for phosphorus analysis.

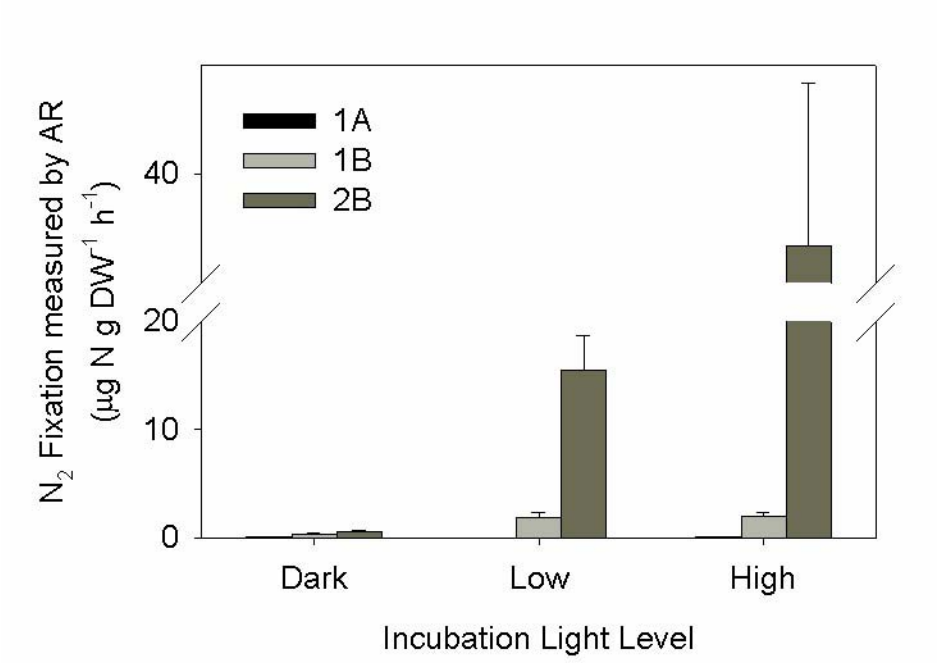

Figure 2. N_2 fixation measured by acetylene reduction (expressed in hourly units) at each incubation light level for the entire study. Bars indicate mean rate determined at each site for the entire summer (± SE).

periphyton P content at site 2B in July (Table 2). In September, C:P and N:P were greatest at site 1A, followed by site 2B then site 1B (Figure 5). Again, these differences were primarily the result of the relatively large difference in periphyton P content observed between sites (Table 2). The N:P ratio in periphyton was not strongly related to the ratio of DIN:SRP in July but appeared to be more positively correlated in September (Figure 4 and Table 1).

Contribution of N_2 fixation to total N uptake

Estimates of the contribution of N_2 fixation to total N uptake determined by AR assays and $\delta^{15}N$ composition of periphyton are shown in Figure 5. In general, $\delta^{15}N$ derived values tended to overestimate the percent contribution of N_2 fixation as measured via AR. Although a strong correlation between estimates existed in May ($r^2 = 0.88$; Figure 5), $\delta^{15}N$ derived values were 1.4 times greater than estimates derived from AR. Furthermore, the correlation between these estimates was greatly reduced in July and September. The poor correlation was the result of greatly reduced estimates of the percent contribution of N_2 fixation determined via AR (0 – 30%) but relatively high estimates from the $\delta^{15}N$ derivation, particularly from site 2B (Figure 5).

Interestingly, both AR and $\delta^{15}N$ derived estimates demonstrated unique relationships with periphyton N content (Figure 6). Periphyton N content and AR derived estimates appeared to correlate on a temporal scale. In particular, AR derived estimates and periphyton N content were positively correlated in May, but negatively correlated in July and September (Figure 6A). Percent contribution of N_2 fixation derived from $\delta^{15}N$ appeared to correlate with periphyton N content on a temporal and spatial scale. At site 1B in May, the relationship between periphyton N

content and percent contribution of N_2 fixation derived from $\delta^{15}N$ was positive and could be modeled on the same regression line with observations from all months at site 2B (Figure 6B).

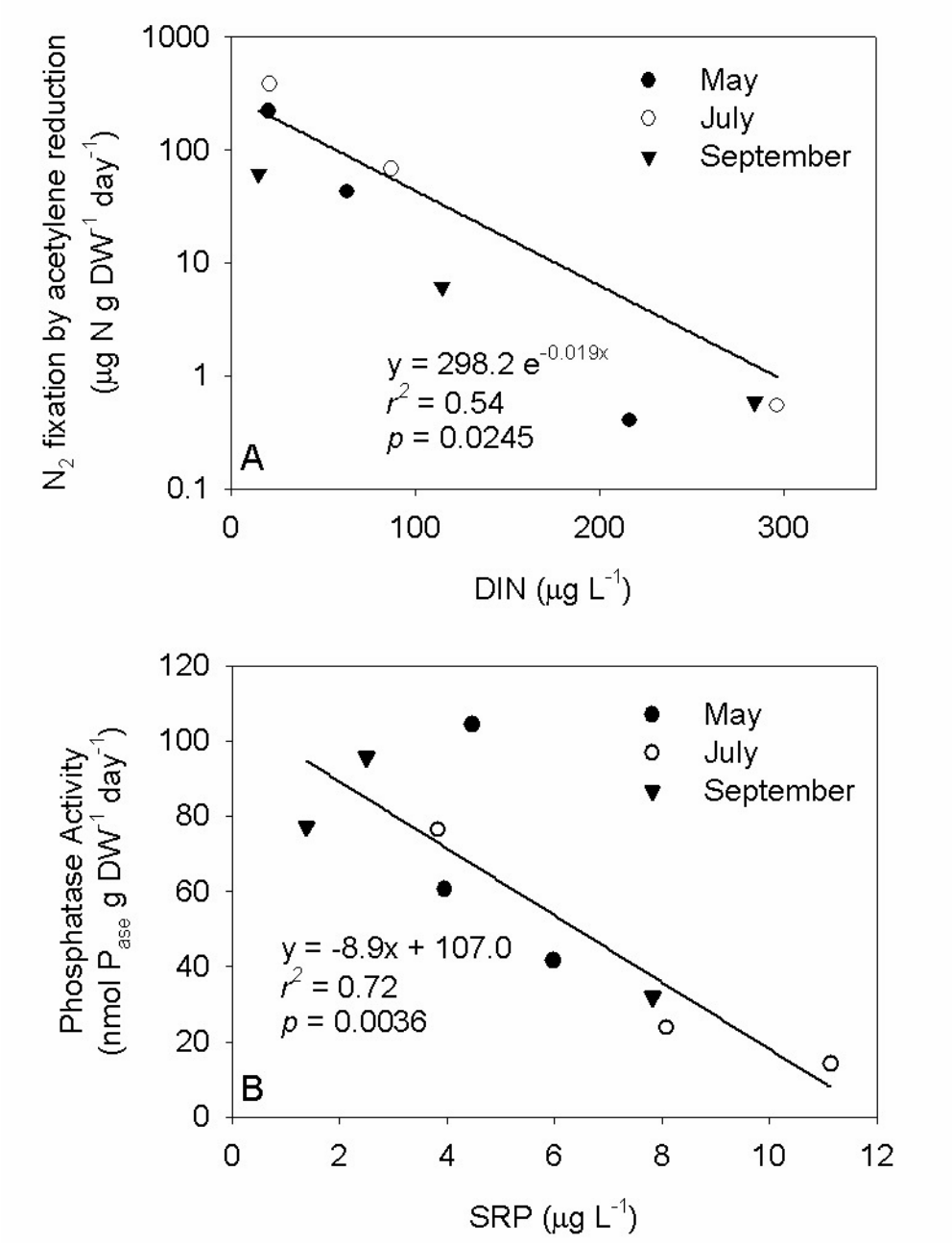

Figure 3. Enzymatic activity versus nutrient concentrations for each sampling event. A) Daily N_2 fixation, measured by acetylene reduction, versus mean DIN concentration in the water column. B) Phosphatase activity versus mean SRP concentration in the water column.

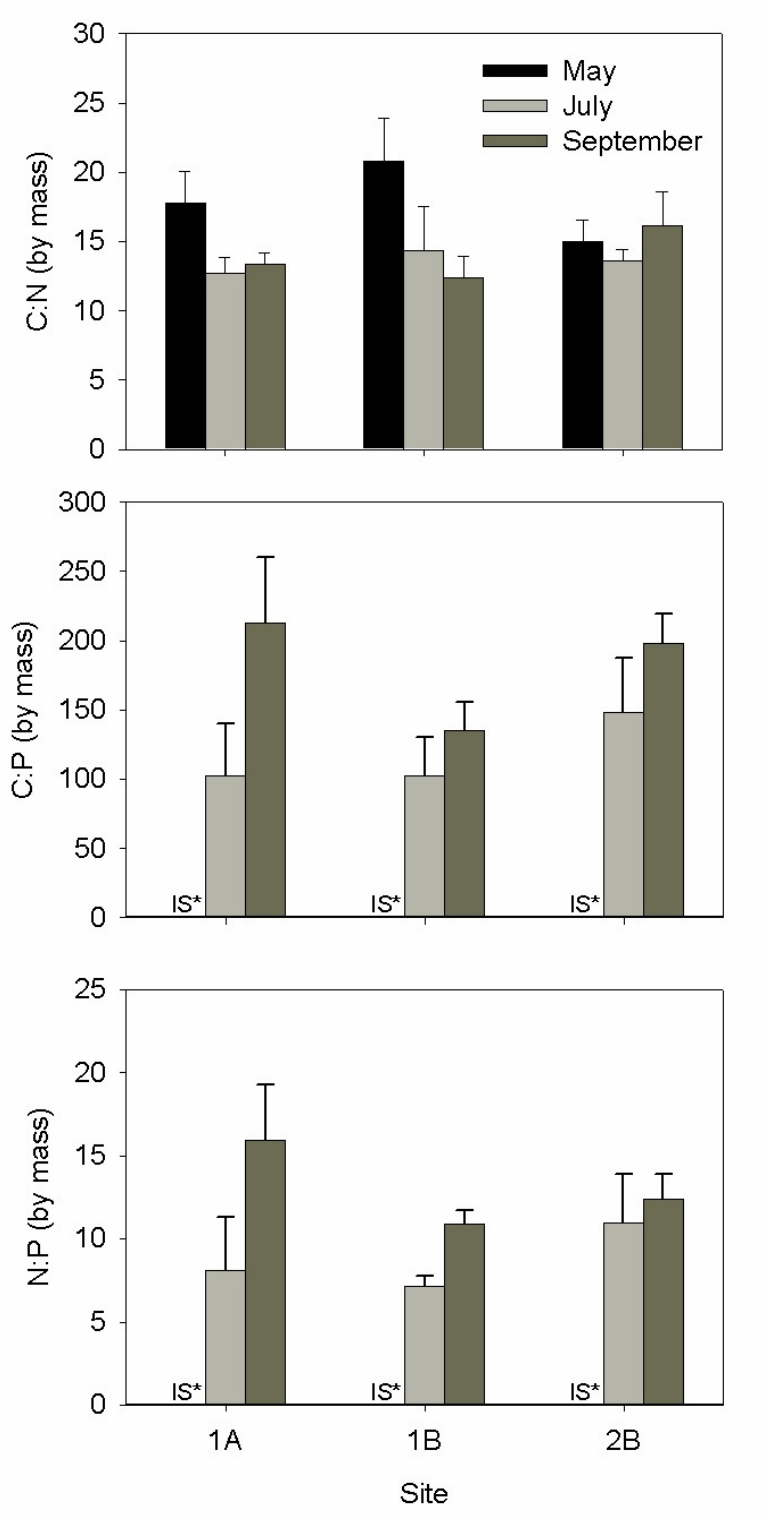

Figure 4. Elemental ratios of periphyton for each sampling event (mean ± SD; n = 7-8, see Table 2 for details on number of samples). Black bars represent samples collected in May 2004, light gray bars represent samples collected in July 2004, and medium gray represent samples collected in September 2004. Insufficient sample was collected during May 2004 for phosphorus determination (IS*), therefore, C:P and N:P values are not available for that sampling event.

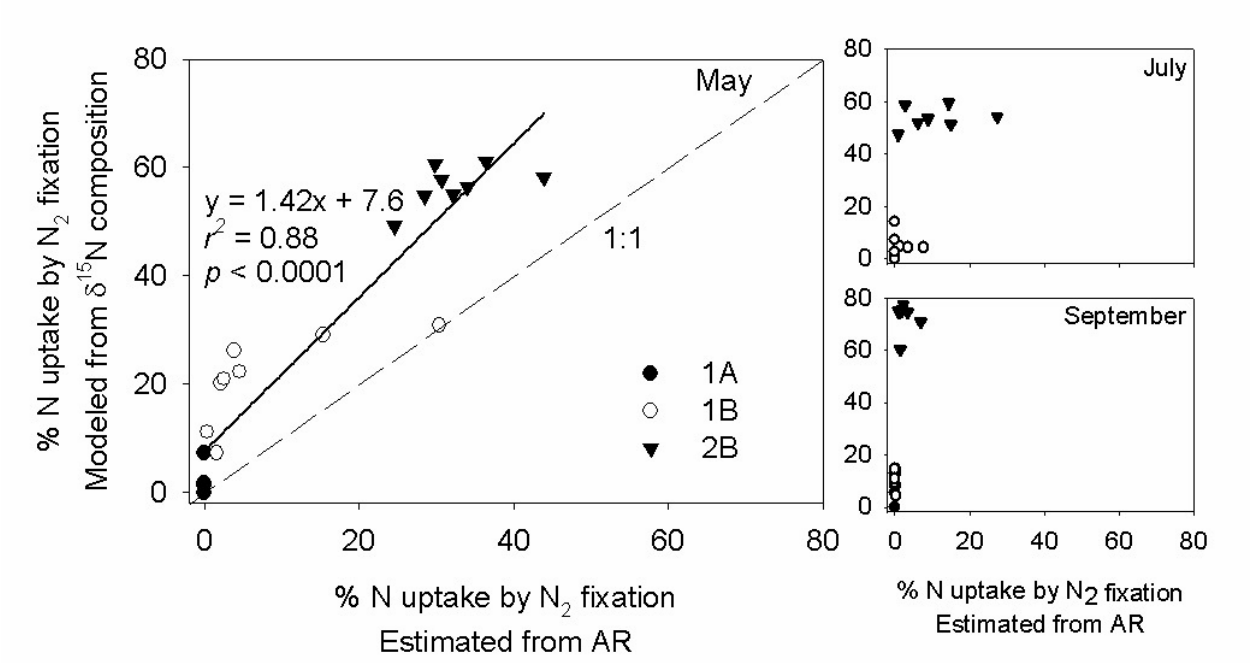

Figure 5. Measured versus modeled percentage of N uptake derived from N_2 fixation. Measured values determined by dividing acetylene reduction estimates of N_2 fixation by estimates of gross N uptake. Modeled values determined from isotope mixing model. Black circles represent samples from area 1A, white circles represent samples from area 1B, and black triangles represent samples from 2B. The mixing model estimates generally agreed with empirical estimates from acetylene reduction in May only. In July and September, modeling with $\delta^{15}N$ tended to overestimate the instantaneous contribution of N_2 fixation to total N uptake.

The correlation between these variables was less pronounced at site 1B in July and September and at site 1A during all sampling events (Figure 6B).

Correlates of Periphyton Primary Production

In May, periphyton primary production appeared to correlate with water column DIN concentrations (Tables 1 and 2). However, this relationship did not exist in July or September. In these months, periphyton primary production was more generally correlated (inversely) with phosphatase activity, and also periphyton P content in September (Table 2). When normalized to periphyton N content and considered over the entire summer, periphyton primary production was strongly inversely correlated with phosphatase activity (Figure 7).

Discussion

Results of this study demonstrate the seasonal evolution of P-limitation in a lentic periphyton community. Periphyton primary production appeared to be N-limited during spring but became P-limited, and remained so, throughout the summer. This was indicated by several relationships between primary production and indicators of nutrient stress. For instance, average primary production was inversely correlated with average DIN in May but became more generally correlated with phosphatase activity in July and September (Tables 1 and 2). This switch

between DIN correlation and phosphatase correlation with primary production

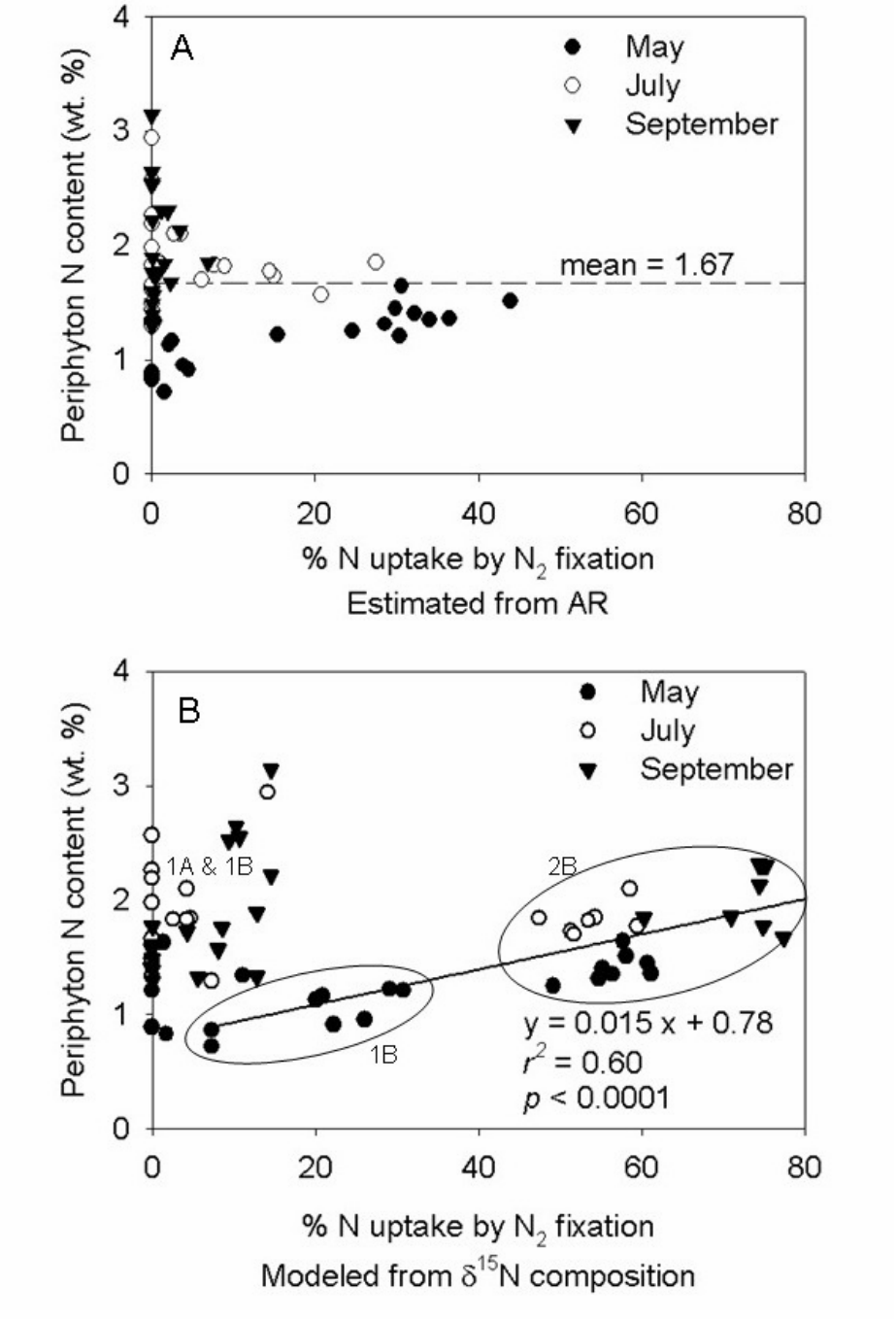

Figure 6. Relationship between N_2 fixation and periphyton nitrogen content. In both plots, black circles represent samples collected in May 2004, white circles represent samples from July 2004, and black triangles represent samples from September 2004. A) Relationship between N_2 fixation measured using acetylene reduction and periphyton nitrogen content in sampling areas 1B and 2B. B) Relationship between N_2 fixation as indicated by $\delta^{15}N$ natural abundance and periphyton nitrogen content in sampling areas 1B and 2B. Decreasing $\delta^{15}N$ corresponds to increasing N_2 fixation. Regression lines were derived for two data subsets: 1) all samples

collected in area 2B and samples collected in area 1B in May 2004, and 2) samples collected in area 1B in July 2004 and September 2004. See discussion for details of these groupings.

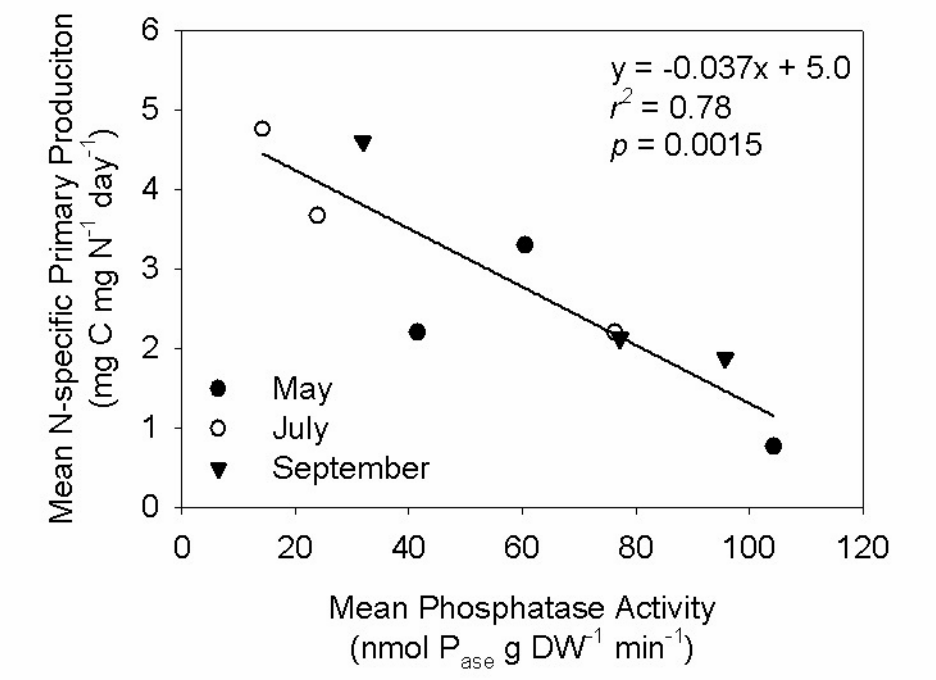

Figure 7. Relationship between mean phosphatase activity and mean nitrogen-specific primary production from all sampling events.

corresponded to a 1.5 to 1.6 fold increase in average periphyton N content from May to July and September, respectively (Table 2). Furthermore, the percent contribution of N_2 fixation to total N uptake, estimated via AR, was generally highest in May and decreased iteratively in July and September. This suggests that periphytic cyanobacteria may have down regulated N_2 fixation in response to accumulating N. In fact, this trend is supported by the N isotopic signature of the periphyton mat. Periphyton $\delta^{15}N$ composition retained the atmospheric signature (approaching 0 ‰) at site 2B even when N_2 fixation measured by AR had diminished (Table 2). However, lighter isotopic values, and subsequently larger isotopic estimates of the percent contribution of N_2 fixation to total N uptake (> 20 %), were generally correlated with higher periphyton N content (Figure 6B). We submit that the accumulation and retention of N in the periphyton mats eventually resulted in P-limited primary production. Interestingly, when primary production was normalized to N content, a strong inverse correlation with phosphatase activity was apparent across all sampling events (Figure 7). Therefore, short-term correlates of nutrient limitation (instantaneous from each month) indicate a springtime period of N-limitation followed by an extended period of P-limitation. However, over a seasonal time scale (all months), N-specific primary production appeared limited by P availability (Figure 7).

Elemental balance within the periphyton mats may also indicate nutrient limitation status. Periphyton C:N was only elevated at sites 1A and 1B in May, and appeared relatively balanced during the remainder of the growing season (Figure 4). Because stoichiometric theory tells us that nutrient limitation of photoautotrophs is generally coupled with an increase in the C:nutrient

ratio (Sterner and Elser 2002), periphyton C:N indicate that N may only have been limiting in May. Furthermore, C:P appears to have increased at all sites from July to September. Unfortunately, it remains unknown whether C:P would have been lowest in May because insufficient sample was available for the determination of P content.

The accumulation and retention of N appears to be a critical factor in controlling primary production and inducing P-limitation. Nitrogen availability was primarily driven by DIN supply and secondarily driven by N_2 fixation. For instance, N_2 fixation was never observed nearest inflowing waters (site 1A) where DIN was relatively high. However, N_2 fixation appeared increasingly important at downstream sites where DIN concentrations were greatly reduced (Tables 1 and 2; Figure 3A). Furthermore, N_2 fixation appeared to balance periphyton N content on the scale of weeks in the downstream, most N poor, areas.

Estimates of the percent contribution of N_2 fixation to total N uptake can be derived using both AR (coupled with estimated of gross N assimilation) and periphyton $\delta^{15}N$ composition. Recent studies have attempted to characterize the importance of lentic periphyton N_2 fixation using the N isotopic method with somewhat mixed results. Inglett et al. (2004) found that average AR rates correlated well with average $\delta^{15}N$ composition in an Everglades periphyton community. However, measurements in that study were limited to one location over a two-year period and potential differences between individual measurements (AR versus $\delta^{15}N$) were not considered. Rejmánková et al. (2004) did use individual measurements in an attempt to predict N_2 fixation rates (derived using AR) from $\delta^{15}N$ signatures in a number of tropical wetland periphyton communities. Although their correlation showed some promise, the dataset had poor resolution when N_2 fixation rates were low. The authors ultimately found that a simple threshold in periphyton $\delta^{15}N$ was the most robust indicator of either high or low N_2 fixation.

In this study, we used a mechanistic N isotope approach (*i.e.* a two-end member mixing model) to develop estimates of the percent contribution of N_2 fixation to total N uptake. When compared with empirical estimates of the percent contribution of N_2 fixation (from AR), $\delta^{15}N$ estimates tended to overestimate the importance of N_2 fixation. Although a good correlation between estimates was observed in May samples, the isotope model overestimated the percent contribution of N_2 fixation to total N uptake by almost 1.5 fold (Figure 5). Furthermore, isotope model values for site 2B were grossly overestimated in July and September (Figure 5). We propose that this overestimation was caused by the accumulation of atmospherically derived N in the periphyton mat through time. This accumulation violated the model assumption of negligible N recycling and therefore caused overestimates of the percent contribution of N_2 fixation. Although not explicitly measured in this study, periphyton communities are known to retain and recycle nutrients with great efficiency (Borchardt 1996). Because instantaneous N_2 fixation (AR) appeared to contribute a large amount of N to periphyton at site 2B in spring and early summer (Figure 6A), accumulation and recycling of this fixed N may have caused periphyton $\delta^{15}N$ to continually reflect a high contribution of N_2 fixation throughout the summer even though instantaneous N_2 fixation rates (AR) had diminished (Figure 5). Therefore, periphyton $\delta^{15}N$ comprises a time-integrated estimate of the relative contributions of DIN and N_2 fixation to the N content of the community. Although this inherent characteristic was suggested in early comparisons between N_2 fixation measured by AR and estimates from $\delta^{15}N$ composition (Gu and Alexander 1993; France et al. 1998), recent studies have not considered this potential

inconsistency and subsequently have generated mixed results on the usefulness of the technique (Rejmánková et al. 2004; Inglett et al. 2004).

Interestingly, relatively low $\delta^{15}N$ (*i.e.* relatively high percent contribution of N_2 fixation total N uptake) was always correlated with periphyton N content when instantaneous N_2 fixation (measured by AR) was contributing to N content. For example, periphyton N at site 2B during all months and at site 1B in May increased by 0.015 % for every 1 % increase in the contribution of N_2 fixation to total N uptake (see regression in Figure 6B). This trend was not apparent at site 1B in July and September and never apparent at site 1A. This evidence in particular, along with reduced N_2 fixation (AR) observed with increasing periphyton N content, suggest the importance of accumulating atmospherically derived N to alleviate N deficiency in this floating periphyton mat. Although a N rich lifestyle could be expected when N_2 fixers are present (Gu and Alexander 1993), our results indicate that fixed N may be efficiently retained by a periphyton community long after instantaneous N_2 fixation ceases.

Conclusions

Results of this study suggest that periphyton communities can rapidly fix and accumulate significant amounts of atmospheric N_2, thereby driving the community toward P-limitation. It appears that the buildup and retention of N to a critical threshold can occur within a matter of weeks during the early growing season, at least within the warm-temperate zone similar to this study. When N content exceeds a critical threshold, instantaneous N_2 fixation rates fall rapidly but isotopic composition continues to reflect an atmospheric N source. We hypothesize that sustained $\delta^{15}N$ in the range of atmospheric N_2 was caused by the efficient retention and recycling of fixed N_2. Efficient retention brought periphyton N content into stoichiometric balance causing N-specific primary production to be limited by P availability. This study confirms the importance of fixed N_2 as a N source to periphyton community production and provides an example of seasonally-evolving P-limitation in a shallow aquatic ecosystem.

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Awards

First prize, student paper competition. Society of Wetland Scientists South Central Chapter Meeting, October 2005, San Marcos, Texas. Paid travel expenses to the SWS 2006 International Congress in Cairns, Queensland, Australia.